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**True digestibility of phosphorus determined by regression method for rapeseed meal**

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## ABSTRACT

One-hundred and forty-four male broiler chickens at 26 d old were employed to determine the ileal true phosphorus digestibility of a rapeseed meal (RSM). The broiler chickens were brooded together and received diets meeting nutrient recommendations from d 0 to 21. On d 21, the broiler chickens were weighed and allocated to three treatments (eight replicates and six broiler chickens per replicate) in a randomized complete block design. The three diets were maize starch and dextrose-based semi-purified diets in which RSM was added at the rates of 450, 560, and 670 g/kg as the sole source of P. All the broiler chickens were euthanised on d 26 and digesta from the distal ileum were collected for chemical analysis. Apparent ileal P digestibility tended to increase linearly ( $P = 0.074$ ) with increasing level of RSM in the diet. Intake of total and ileal digestible P, as well as total P output, increased linearly ( $P < 0.01$ ) with increasing dietary P supplied by RSM. Regression of P output against dietary P gave the regression equation:  $Y = 0.575x + 1.140$  with the estimate of true P indigestibility being 57.5%. Consequently the ileal true P digestibility for the RSM was calculated to be 43%. It was concluded that RSM can be a substantial P source along with its use as a source of protein and energy for broiler chickens.

**Keywords:** Broiler chickens, Ileal, Phosphorus, Rapeseed meal, True digestibility

## 1. Introduction

Poultry feeds are formulated using mainly cereal grains and oilseed meals. Efficient utilization of these raw materials depends on an accurate understanding of their nutritional value. Phosphorus is one of the most nutritionally important minerals and expensive ingredients in poultry diets. Much of P in plant feedstuffs is bound to phytate that is less available to poultry (Broz and Ward, 2007; Summers et al., 1983). Therefore, inorganic P is supplemented to poultry

diets to meet P requirement. Because of the confusion that can result from using multiple terminologies to define dietary P and its utilization, WPSA (2013) suggested using digestible P for assessing dietary P and provided a protocol for estimating digestible P in feedstuffs. Few studies have provided information about apparent P digestibility in rapeseed or rapeseed meal (RSM) and true P digestibility (TPD) of canola meal for pigs (Akinmusire and Adeola, 2009; Rodehutscord et al., 1997) but there is a dearth of such information for poultry. Therefore the objective of the current study was to determine TPD in a RSM.

## **2. Materials and Methods**

All the animal experiment procedures used in the current study were approved by the Scotland's Rural College's Animal Experiment Committee. A total of 144 Ross 308 broiler chickens were used for the experiment. The broiler chickens were brooded together in a floor pen from hatch to 21 d of age during which time they received a standard commercial diet that meets Ross 308 nutrient specification (<http://en.aviagen.com/ross-308/>). On d 21, broiler chickens were weighed and allocated to three treatments in a randomized complete block design. Each treatment had eight replicates and six broiler chickens per replicate. The ingredient and chemical compositions of the experimental diets are shown in Table 1. The three semi-purified diets had titanium dioxide as an indigestible marker, and contained graded levels of RSM as the only source of P. The graded levels of RSM resulted in increasing level of total P in the three diets. The diets were fed for 5 d, the broiler chickens were euthanized on d 26 and digesta were collected from distal half of the ileum (WPSA, 2013).

Diets, RSM, and ileal digesta were analyzed, as appropriate, for dry matter, N, minerals, Ti, phytate P, and gross energy using AOAC (2006) methods. Minerals were analyzed using inductively coupled plasma – optical emission spectroscopy (Method 990.08; AOAC, 2006)

following digestion, in turn, in concentrated  $\text{HNO}_3$  and  $\text{HCl}$ . Glucosinolate in RSM was analyzed using ISO method 9167-1 (ISO, 1992).

The apparent digestibility data (calculated using the index method) were analyzed by the GLM procedure of SAS 9.3 (SAS, 2011). Linear and quadratic effects of RSM inclusion levels on all P utilization responses were evaluated using orthogonal polynomial tests. Phosphorus intake (g/kg DM) and P output (g/kg DM) were calculated as described previously (Adebiyi and Olukosi, 2015), and  $P_{DMO}$  (g/kg DM output) data were regressed against P intake (g/kg) using REG procedure of SAS 9.3. Ileal TPD value was derived as described previously (Adebiyi and Olukosi, 2015).

### 3. Results

The analyzed total P in the diets (Table 1) showed that the expected dietary P levels were met. The RSM contained (g/kg DM) 370, 10.2, 5.8, and 8.1 of crude protein, total P, phytate P, and Ca, respectively. In addition glucosinolate content for the RSM was 11.2  $\mu\text{m/g}$  whereas sinapine and tannin contents were 4.7 and 2.11 mg/g, respectively. The analysis showed that approximately 57% of total P in the RSM is phytate P and that glucosinolate, sinapine, and tannin levels were within the levels expected of the meals from modern varieties of rapeseed.

Table 2 shows the data for apparent digestibility of the experimental diets. Ileal DM digestibility decreased linearly ( $P < 0.01$ ) with increasing level of RSM in the diets. There was linear increase ( $P < 0.01$ ) in ileal digestible P, total P intake, digestible P intake, and P output with increasing dietary level of RSM. Apparent P digestibility tended to increase linearly ( $P = 0.07$ ) with increasing dietary level of RSM. There were no quadratic effects of increasing RSM level in the diet on any of the measured responses.

The regression of dietary P (g/kg DM) against P output (g/kg DM output) produced the linear equation:  $Y = 0.575x + 1.140$ . The slope of the regression equation ( $b$ , 0.575) is an estimate of coefficient of P indigestibility and the intercept 1.14 is an estimate of the endogenous P loss (g/kg DM intake). The standard error of the linear term was 0.124 and that of the intercept was 0.825 with  $r^2$  of 0.61. Coefficient of TPD was calculated as:  $1 - 0.575$ , and gave a value of 0.425 (or 43%). True digestible phosphorus for the RSM was derived as the product of total P content (10.2 g/kg DM) of the RSM and its TPD coefficient (0.43) and gave a value for true digestible P of 4.39 g/kg (DM).

#### 4. Discussion

The objective of the current study was to determine the TPD of an RSM for broiler chickens by means of the regression method using protocol similar to that developed by WPSA (2013), with the exception that egg albumin was not used in the diets fed in the current experiment. The value for TPD estimated for the RSM in the current study is 43%. The value is close to, though numerically lower than, the 47% estimated for canola meal by Mutucumurana et al. (2014). In pigs, Akinmusire and Adeola (2009) reported a TPD of 34% for canola meal whereas Rodehutsord et al. (1997) reported apparent digestibility values of 42 or 24% for full fat rapeseed or solvent extracted rapeseed meal, respectively.

The level of total P in the RSM investigated in the current study was 10.2 g/kg and the phytate P was 5.8 g/kg thus the non-phytate P is 4.4 g/kg or 43% of total P. The true ileal digestible P (calculated as product of coefficient of TPD and total P in the RSM) was 4.39 g/kg. Consequently the total digestible P (4.39 g/kg) was virtually the same as total non-phytate P (4.4 g/kg) content in the meal. It was assumed, for the calculations of total digestible P above, that all

the non-phytate P in the RSM was absorbed. This is probably not true, and hence it is possible that the contribution of phytate-P to the total digestible P in the sample was greater than zero value suggested by the calculations above. In all probability, a small portion of the phytate-P in the RSM contributed to the true digestible P (4.39 g/kg) calculated above. This suggests that very little phytate-P was digested, and hence there is considerable opportunity for phytase to liberate P from phytate in RSM.

The methodology used for calculating TPD in the current study is the approach proposed by Dilger and Adeola (2006), which involves estimation of true P indigestibility from the slope of the regression of P output (g/kg DM output) against dietary P level. By definition, TPD is calculated as 1 minus true P indigestibility. On the other hand, TPD can be calculated directly from the slope of regression of digestible P intake (g/kg DMI) against dietary P level as suggested by WPSA (2013). Because the data used to generate digestible P intake can also be used to calculate P output, it is possible to run the two regression analyses and compare the outputs; and by definition, the slope of one method should be 1 minus the slope of the other method; whereas the intercept should be the same, although with different signs.

A requirement for using linear regression analysis to estimate true digestibility is that there must be a statistically significant regression coefficient and it is important to ensure that this requirement is met before proceeding further with the use of linear regression. However, an inherent issue with regression analysis is the possible presence of outlying observations or influential data points. Instances of such data points can produce erroneous estimates of true digestibility and EPL and thus make comparison across studies difficult. It is therefore important to ensure that data are checked for such issues before proceeding with the use of linear regression.

Regression of P output against dietary P in the current study gave an estimate of EPL as 1.14 g/kg DMI (standard error was 0.83), whereas regression of digestible P intake against dietary P gave an EPL estimate of -1.14 g/kg DMI. The standard error of EPL estimate was relatively large and this might be responsible for the relatively wide confidence interval and the lack of statistical significance. It would be useful in future estimates to indicate whether these estimates of EPL are different from zero especially in cases where enzymes supplementation improved digestibility of the nutrient. This will help indicate how much of the improvement in digestibility in response to the enzyme was due to reduction in EPL. For example, Akinmusire and Adeola (2009) showed that phytase supplementation increased TPD of canola meal from 34 to 61% and decreased EPL estimate from 101 to 38 mg/kg DMI. At first glance, there appears to be a drastic reduction in EPL due to phytase supplementation but, as the authors pointed out, the estimates were not different from zero and hence it could be concluded that the improvement in TPD as a result of phytase supplementation was not driven primarily by a reduction in EPL.

## **5. Conclusion**

It is concluded from the current experiment that the coefficient of ileal TPD for the RSM tested is 0.43. Therefore, less than half of the total P in the RSM was digested at the ileal level and, consequently, there is potential for further P digestibility with phytase supplementation. In view of the above, RSM can serve as a P source in addition to being a source of protein and metabolisable energy for broiler chickens.

## **Conflict of interest**

We confirm there was no conflict of interest.



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187 **Table 1**

188 Ingredient composition (g/kg) and calculated analysis of the experimental diets (g/kg as fed).

Item	Diet rapeseed meal content		
	450 g/kg	560 g/kg	670 g/kg
Rapeseed meal	450	560	670
Maize starch	406	294	182
Dextrose	100	100	100
Soybean oil	20	20	20
Limestone	7	9	11
DL-Met	2	2	2
Vitamin- trace mineral premix <sup>a</sup>	10	10	10
Titanium dioxide	5	5	5
Total	1000	1000	1000
Chemical composition, calculated (g/kg, dry matter basis)			
Dry matter (analysed)	911	921	926
Crude protein	188	231	275
Ca	5.9	7.4	8.9
Total P <sup>b</sup>	5.0	6.2	7.4
Phytate P	2.9	3.5	4.2

189 <sup>a</sup> Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 IU;  
190 vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic  
191 acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg;  
192 thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11  
193 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

194 <sup>b</sup> Analysed total P was 5.6, 6.7, and 7.6 g/kg (DM) for diets with RSM contents of 450,  
195 560, and 670 g/kg, respectively

196 **Table 2**

197 Ileal nutrient digestibility by the broiler chickens receiving the experimental diets.

Item	Diet rapeseed meal content			Pooled SEM	Contrasts	
	450 g/kg	560 g/kg	670 g/kg		Linear	Quadratic
Dry matter digestibility	63.3	61.3	57.9	2.1	0.001	0.426
Apparent P digestibility	21.6	26.3	26.9	4.0	0.074	0.362
Digestible P, g/kg DMI	1.21	1.77	2.03	0.15	0.003	0.508
Total P intake, g	17.8	22.1	24.4	0.7	0.001	0.783
Total P intake, g/kg DMI	5.96	6.73	7.56	-	-	-
Digestible P intake, g	3.85	5.76	6.41	0.40	0.004	0.370
P output, g/kg DMI	4.39	4.96	5.52	0.13	0.001	0.487

198 SEM – standard error of the mean; DMI – dry matter intake